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Applied Biosystems® *TrueScience*™ Aneuploidy STR Kits

Software Setup and Data Analysis

User Guide

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About This Guide

Purpose

The *Applied Biosystems® TrueScience™ Aneuploidy STR Kits Software Setup and Data Analysis User Guide* provides recommended procedures for the setup and use of Data Collection Software and GeneMapper® Software for use with the Applied Biosystems Genetic Analyzers listed below:

Genetic Analyzer	Data Collection Software	Data Analysis Software
3130 Genetic Analyzer *or* 3130*xl* Genetic Analyzer	Data Collection Software v3.0	GeneMapper® Software v4.0 and v4.1
	Data Collection Software v3.1	GeneMapper® Software v4.0 and v4.1
3500 Genetic Analyzer *or* 3500xL Genetic Analyzer	3500 Data Collection Software v1.0	GeneMapper® Software v4.1

Prerequisites

This guide assumes a basic knowledge of the use of Applied Biosystems Genetic Analyzers listed in the table above.

This guide assumes a basic knowledge of Data Collection Software and GeneMapper® Software. For a list of additional resources, see "Related documentation" on page 53.

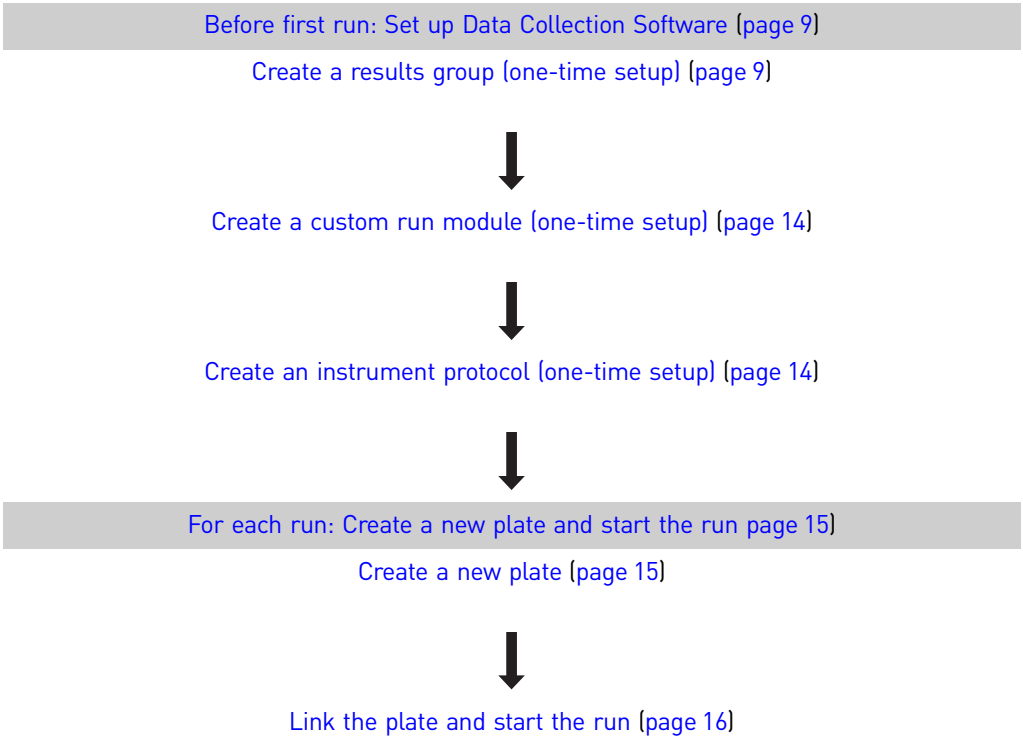
This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

1 Set Up and Use Data Collection Software with Applied Biosystems 3130 Series Genetic Analyzers

Use the procedures in this chapter to set up Data Collection Software v3.1 and v3.0 for data analysis with GeneMapper® Software v4.1 or v4.0.

This chapter covers:

Workflow for Data Collection Software setup



Data Collection Software terms

In the 3130 Series Data Collection Software, each injection is referred to as a "run".

The 3130 Series Data Collection Software uses the elements below to specify settings for data collection.

Data Collection Software Element	Specifies settings for
Instrument protocol	Data collection
Results group	Defines the file type, the file name, analysis type, and file save locations that are linked to sample injections.

Refer to the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* (PN 4352715) for additional information.

Before first run: Set up Data Collection Software

Before setting up samples for the first time, use the following instructions to create a results group and an instrument protocol in the Data Collection Software.

Create a results group (one-time setup)

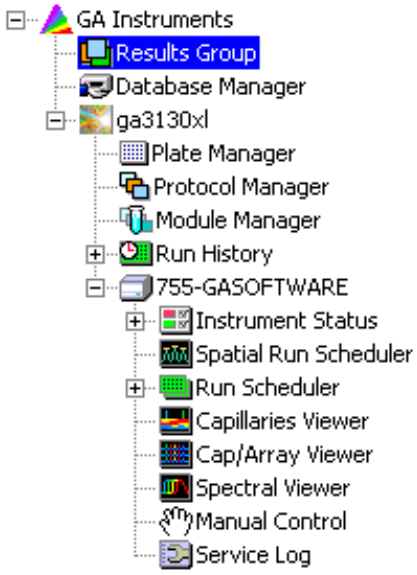
Note: Create one results group as described below if you want to store all of your sample data files in the same folder. If you want to store sample data files from different kits in separate folders, create a results group for each kit (for example, "AN_STR_Plus_Results_Group", "AN_STR_XY_Results_Group", "AN_STR_21_Results_Group", "AN_STR_18_Results_Group", and "AN_STR_13_Results_Group").

1. Select **Start ▸ All Programs ▸ Applied Biosystems ▸ Data Collection ▸ Run 3130 Data Collection**.

   The Services Console opens. After all of the services start, the data collection software opens.

   Note: Access the Help system by pressing **F1**, by clicking in the toolbar of the Data Collection Software window, or by selecting **Help ▸ Contents and Index**.

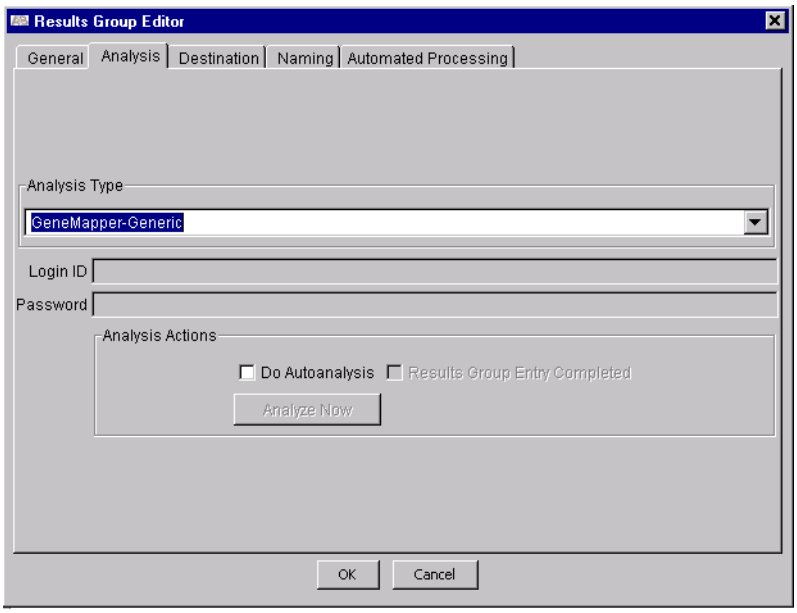
2. Select **Results Group** in the left task pane.



3. In the Results Group Manager, click **New** to create a new Results Group. The Results Group Editor opens.

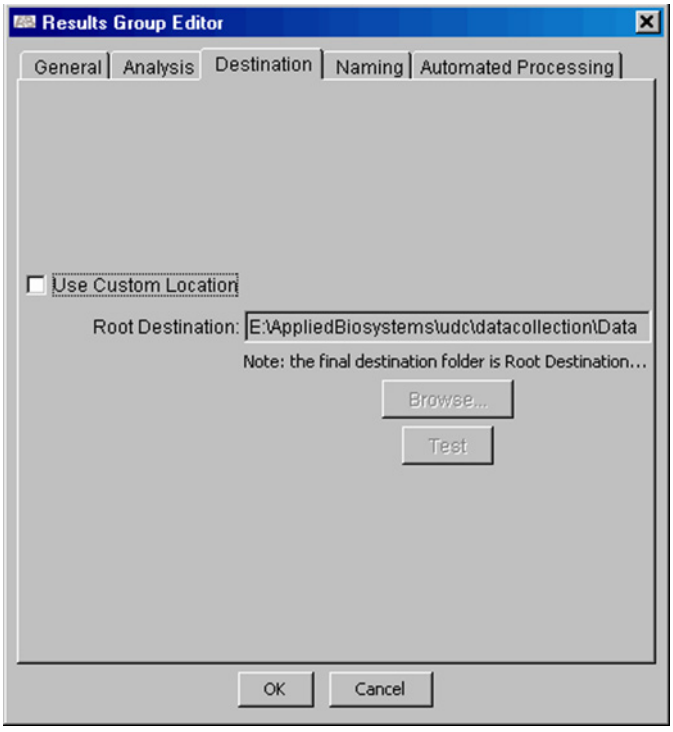
4. In the General tab of the Results Group Editor, enter **AN_STR_Results_Group** for the Results Group Name.

5. In the Analysis tab:
   a. Make sure Do Autoanalysis is not selected.
   b. Select the Analysis Type **GeneMapper-Generic**.

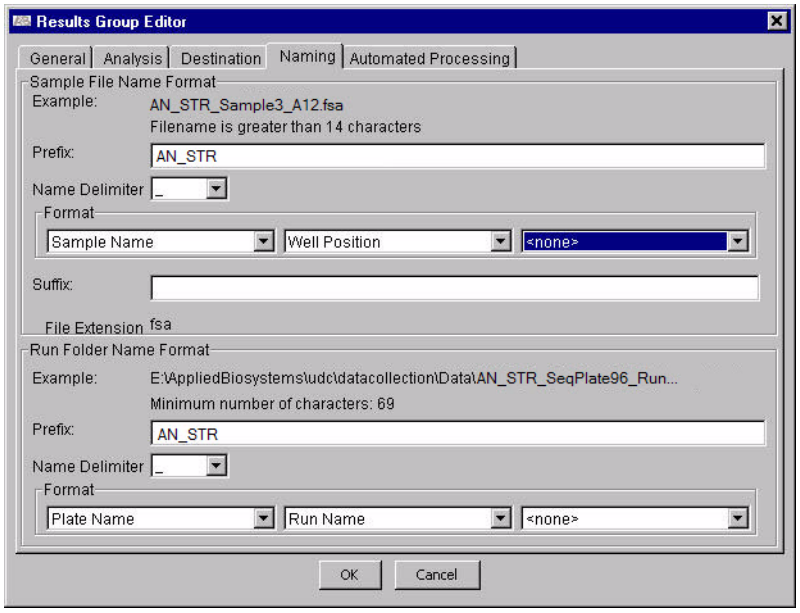


6. In the Destination tab, either:
   - Review the default location where the data will be stored

     or
   - Select **Use Custom Location**, then browse to the location where you want the data to be stored



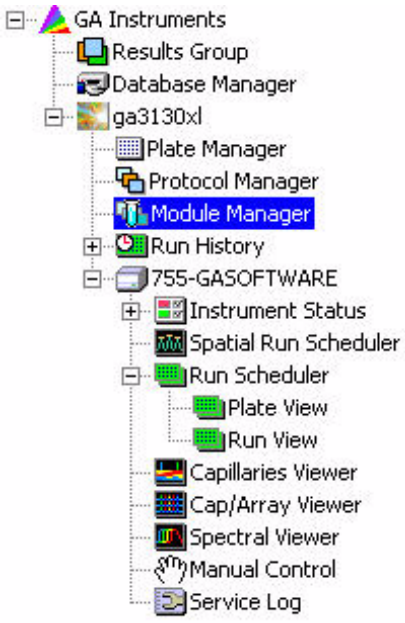
7. In the Naming tab:
   - (Optional) Prefix: Enter **AN_STR** for both Sample File Name and Run Folder Name Format
   - Name Delimiter: Select an option from the drop-down list for both Sample File Name and Run Folder Name Format
   - Format:
     - Sample File Name Format: Select **Sample Name** and other options. For example, select **Well Position**, **Sample Name**, and **Capillary Number.**
     - Run Folder Name Format: Select options, for example, select **Plate Name** and **Date of Run.**



8. Click **OK** to save and exit.

Create a custom run module (one-time setup)

1. Select **Module Manager** in the left task pane.

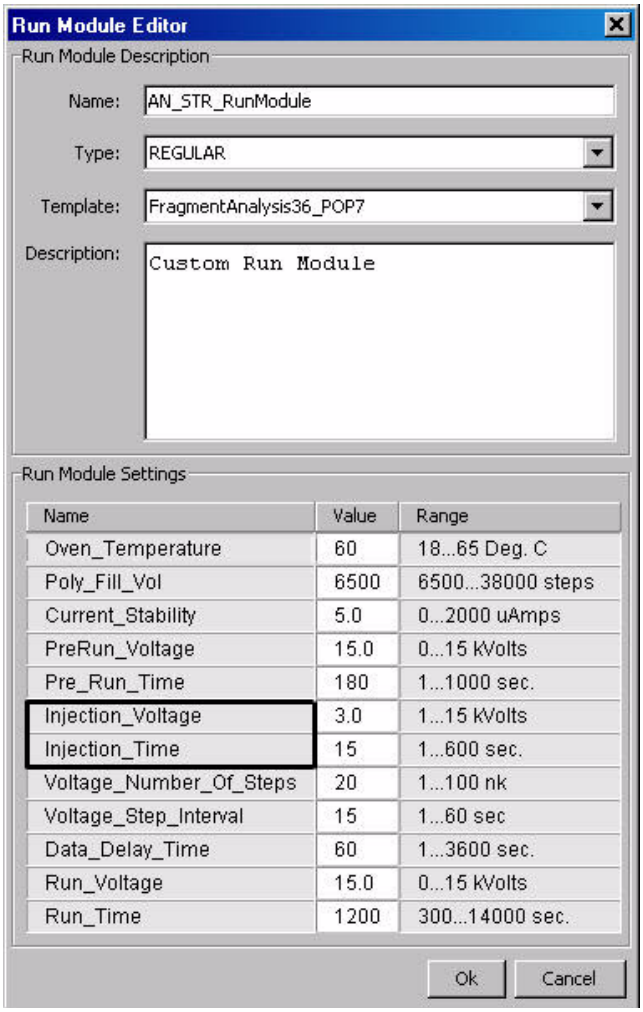


2. In the Module Manager, click **New** to create a new Run Module.

3. In the Run Module Editor, enter the following information:
   - Run Module Description
     - Name: For example, enter **AN_STR_RunModule**

     Note: Make sure to use "_" rather than spaces to avoid triggering an error message.
     - Type: Select **REGULAR**

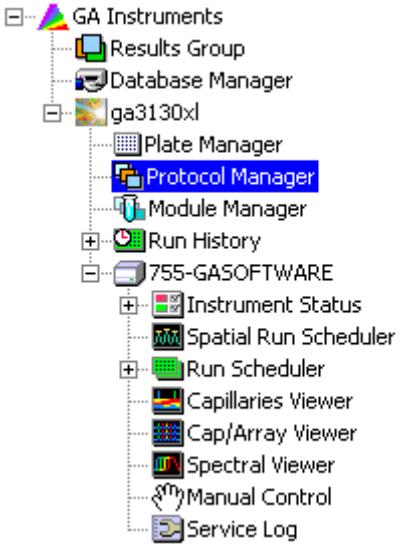
  - Template: Select **FragmentAnalysis36_POP7**
  - Description: (Optional) Enter a description
- Run Module Settings: Change only the following:
  - Injection_Voltage: Enter **3.0 kVolts**
  - Injection_Time: Enter **15 seconds**



4. Click **OK** to save and exit.

Create an instrument protocol (one-time setup)

1. Select **Protocol Manager** in the left task pane.

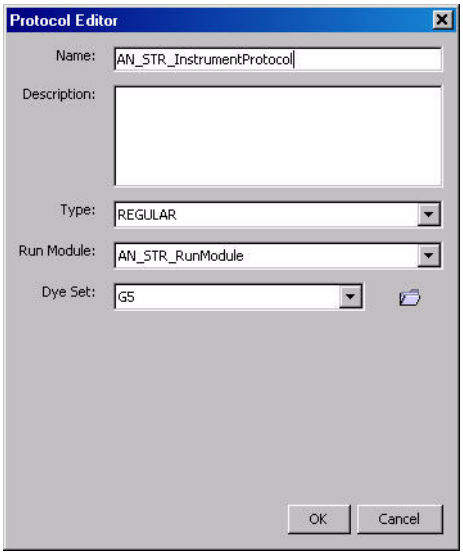


2. In the Instrument Protocol Manager, click **New** to create a new Instrument Protocol.

3. In the Protocol Editor, enter the following information:
   - Name: Enter **AN_STR_InstrumentProtocol**

     Note: Make sure to use "_" rather than spaces to avoid triggering an error message.

   - Description: (Optional) Enter a description
   - Type: Select **REGULAR**
   - Run Module: Select **AN_STR_RunModule**
   - Dye Set: Select **G5**



4. Click **OK** to save and exit.

For each run: Create a new plate and start the run

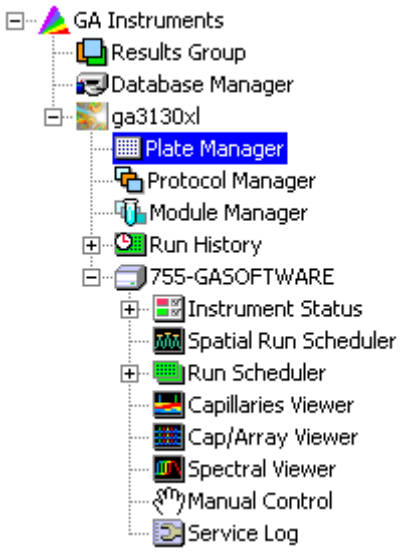
After setting up the software (see "Before first run: Set up Data Collection Software" on page 9), use the following instructions each time you load a reaction plate into the 3130/3130*xL* instrument.

Note: For more information, access the Help system by pressing **F1**, by clicking in the toolbar of the Data Collection Software window, or by selecting **Help ▸ Contents and Index**, or see the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* (PN 4352715).

Create a new plate

1. If the Data Collection Software is not already running, select **Start ▸ All Programs ▸ Applied Biosystems ▸ Data Collection ▸ Run 3130 Data Collection**.
   The Services Console opens. After all of the services start, the data collection software opens.

2. Select **Plate Manager** in the left task pane.



3. At the bottom of the Plate Manager window, click **New** to create a New Plate.

4. In the New Plate Dialog, enter the following:
   - Plate Name: Enter the plate name, for example **AN_STR_Plate**
   - Application: Select **GeneMapper-Generic**
   - Plate Type: Select a plate type
   - Owner Name: Enter the name of the person who set up the plate
   - Operator Name: Enter the name of the person who will run the plate

5. In the New Plate Dialog, click **OK**.
   The GeneMapper Plate Editor opens.

6. In the GeneMapper Plate Editor, enter the following information for each sample:
   - Sample Name: Enter a unique sample name

     Note: Add PLUS, XY, 21, 18, or 13, as appropriate, to the beginning of each sample name to facilitate sorting in GeneMapper® Software.

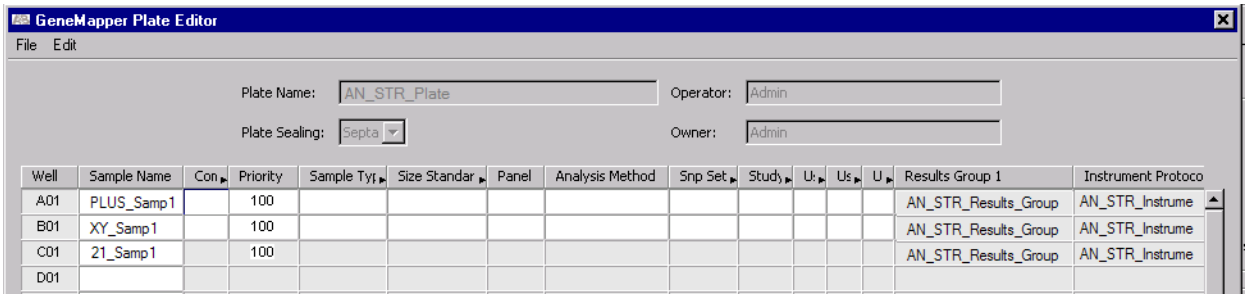
   - Results Group: Select **AN_STR_Results_Group**

     Note: If you created separate results groups for sample data files for each kit, select the appropriate results group for each sample.

   - Instrument Protocol: Select **AN_STR_Instrument_Protocol**
   - Comment, Priority, User-Defined 1, 2, and 3: (Optional) Edit or enter information in these fields

   Note: Leave Size Standard, Panel, and Analysis Method blank. These fields are required with GeneMapper® Software.

   Note: After entering all sample names and selecting the results group and instrument protocol for the first sample, you can highlight the results group and instrument protocol columns, then press **Ctrl+D** (Edit ▸ Fill Down) to copy these selections to the remaining sample rows.



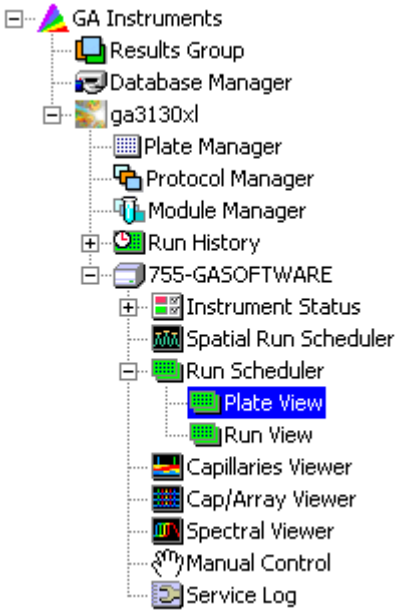
7. Click **OK** to exit and save the plate.

Link the plate and start the run

Note: Before beginning the run, refer to the *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* (PN 4352715) for instructions on checking the system status and checking the consumables status.

1. Prepare the plate with the samples, then load it into the instrument autosampler.

2. Navigate to **Run Scheduler** ▸ **PlateView** in the left task pane.



3. Click **Find All** to find all plates in the Plate View.
4. Select the plate record.
5. Click the plate-position indicator. The plate map color changes from yellow to green when the plate is successfully linked.
6. Click (Start Run) in the tool bar to start the run.
7. In the Process Plates dialog box, click **OK**.

After the run, review the data as described in Chapter 3.

2 Set Up and Use Data Collection Software with Applied Biosystems 3500 Series Genetic Analyzers

Use the procedures in this chapter to set up 3500 Data Collection Software v1.0 for data analysis with GeneMapper® Software v4.1.

This chapter covers:

Workflow for Data Collection Software setup

Before first run: Set up Data Collection Software(page 21)
Import Data Collection Software settings (one-time setup) (page 21)

Edit the plate template (one-time setup) (page 22)

For each run: Create a new plate and start the run(page 25)
Create a new plate from a template (page 25)

Link the plate and start the run (page 27)

3500 Data Collection Software terms

In the 3500 Series Data Collection Software, a "run" refers to all injections in an injection list. An injection is an instance of 8 or 24 samples (depending on instrument configuration) processed simultaneously under the same conditions.

The 3500 Series Data Collection Software uses the elements below to specify settings for data collection. Note that you no longer need to create or select an instrument protocol; it is part of an assay (described below).

New 3500 Series Data Collection Software element	Specifies settings for
Primary analysis (sizecalling) protocol and templates	Sizecalling
File name convention and templates	File naming
Results group and templates	Naming, sorting, and customizing the folders in which sample data files are stored.
Assay and assay templates	Data collection and processing. It contains: • Instrument protocol (dye set and run configuration) • Primary analysis (sizecalling) protocol
Plate template	• Plate parameters • Assay • File name convention • Results group

Before first run: Set up Data Collection Software

Import Data Collection Software settings (one-time setup)

1. Download the Data Collection Software settings:
   a. Go to **www.appliedbiosystems.com/aneuploidy**, then select the **Literature/ Support** tab.
   b. Under Software Downloads, click the **Download Software Settings** link.
   c. Select **AN_STR_DataCollectionv1_0_ Support_Files_Rev1.zip**.
   d. In the File Download dialog box, click **Save**, then save the file to your Data Collection Software computer.
   e. Unzip the file.

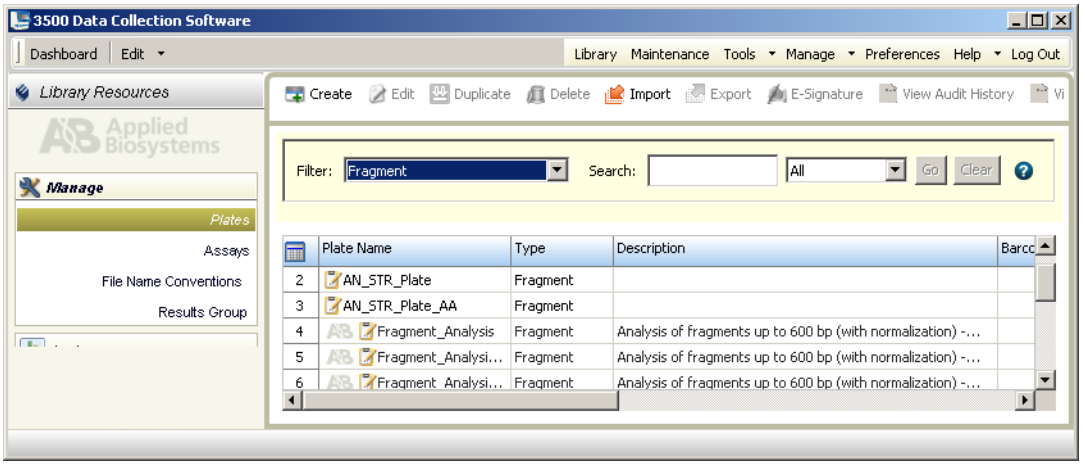
2. Select **Start ▸ All Programs ▸ Applied Biosystems ▸ 3500 ▸ 3500.**

   Note: For complete start-up instructions, refer to the *Applied Biosystems 3500/ 3500xL Genetic Analyzer User Guide* (PN 4401661).

3. In the 3500 Log In dialog box, enter your User Name and Password, then click **OK** to launch the Data Collection Software.

4. Select **Library** in the menu bar to access the Library workflow.

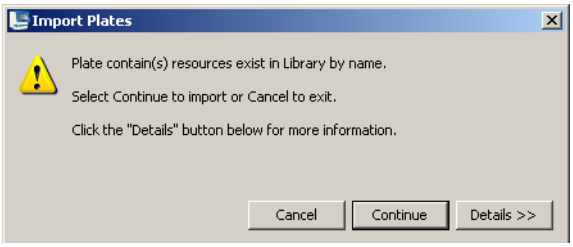
5. Click **Plates** in the left pane.



6. In the Library tool bar, click Import (Import).

7. Navigate to the **AN_STR_DC_Support_Files_Rev1** folder. Locate and add these files:
   - AN_STR_Plate_8cap
   - AN_STR_Plate_24cap

8. Click **Continue** when you see the following message.



Edit the plate template (one-time setup)

Edit the provided plate template to check the settings and to specify the number of plate wells:

1. If necessary, click **Dashboard** to access the dashboard.

2. In the Dashboard, click 

(Create Plate From Template) to display the Open Plate Template from Library dialog box.

3. In the Filter field, select **Fragment**.

4. Select either **AN_STR_Plate_8cap** or **AN_STR_Plate_24cap**, then click **Open**.

5. In the Define Plate Properties screen:

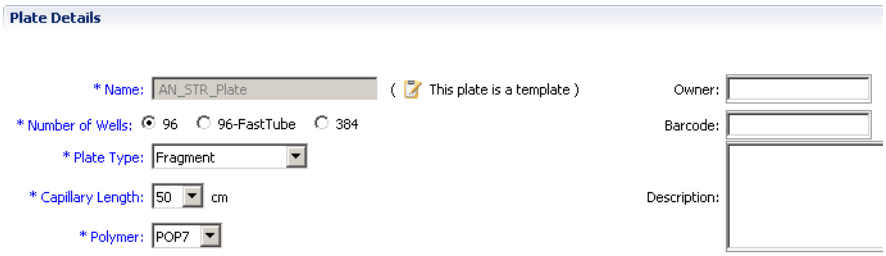
   a. In the name field, rename the plate with a unique name, for example AN_STR_Plate_8cap_template or AN_STR_Plate_24cap_template.

   b. Select the number of wells.

   Note: Select **96** if you are using a 96-well standard reaction plate or 8-strip standard tubes with the appropriate retainers.

   c. Confirm or edit the following plate details:

   - Plate type: **Fragment**
   - Capillary length: **50**
   - Polymer: **POP7**
   - Owner, Barcode, and Description: (Optional) Enter information in these fields



6. In the left pane, click **Assign Plate Contents**.

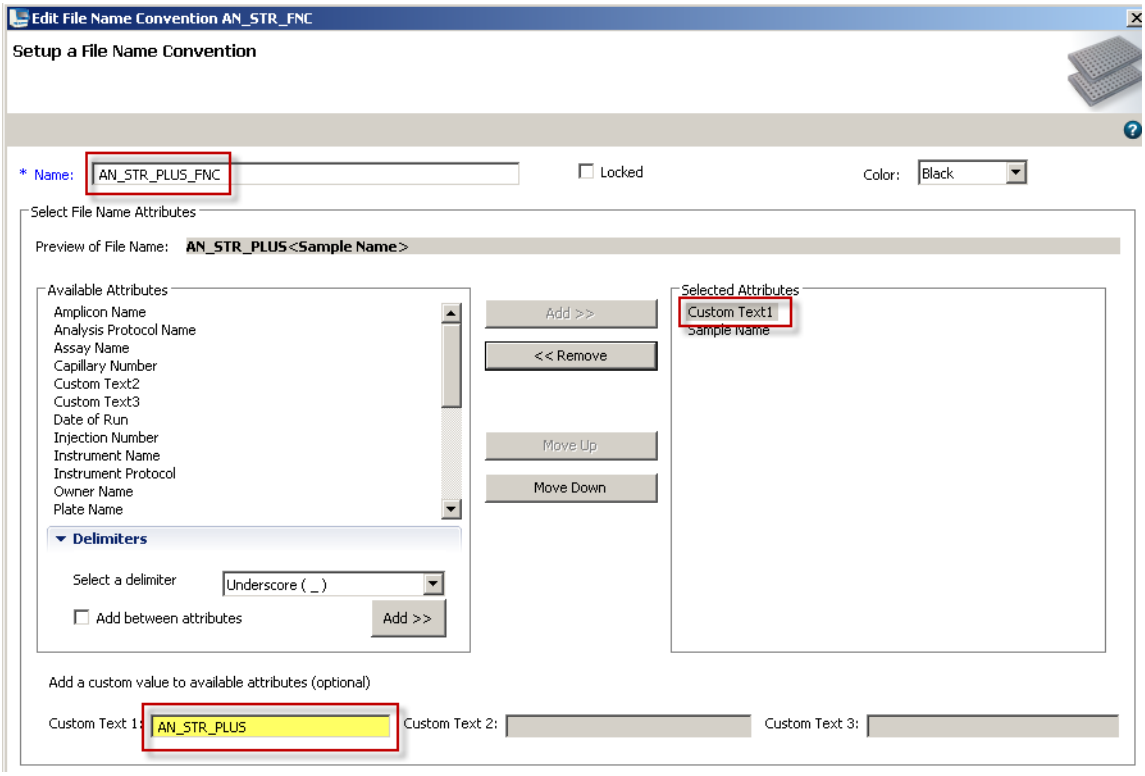
The assays, file name conventions, and results groups associated with the plate template are displayed at the bottom of the Assign Plate Contents screen. Note that one file name convention and one results group are associated with the plate template.

If you will include samples from different kits on the same plate, and you want to specify different naming conventions and storage locations for date files for each kit, create additional file name conventions and results groups for the plate template as described in the next steps.

7. To create new file name conventions and add them to the plate template:

   a. Under File Name Conventions, click **Actions**, then select **New**.

   b. Edit the file name convention as shown below to add a kit prefix to the sample file name.



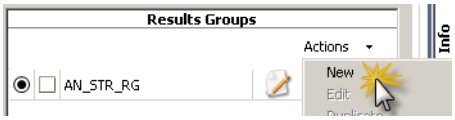
   c. Click **Apply to Plate** (in addition, you can click **Save to Library**), then click **Close**.

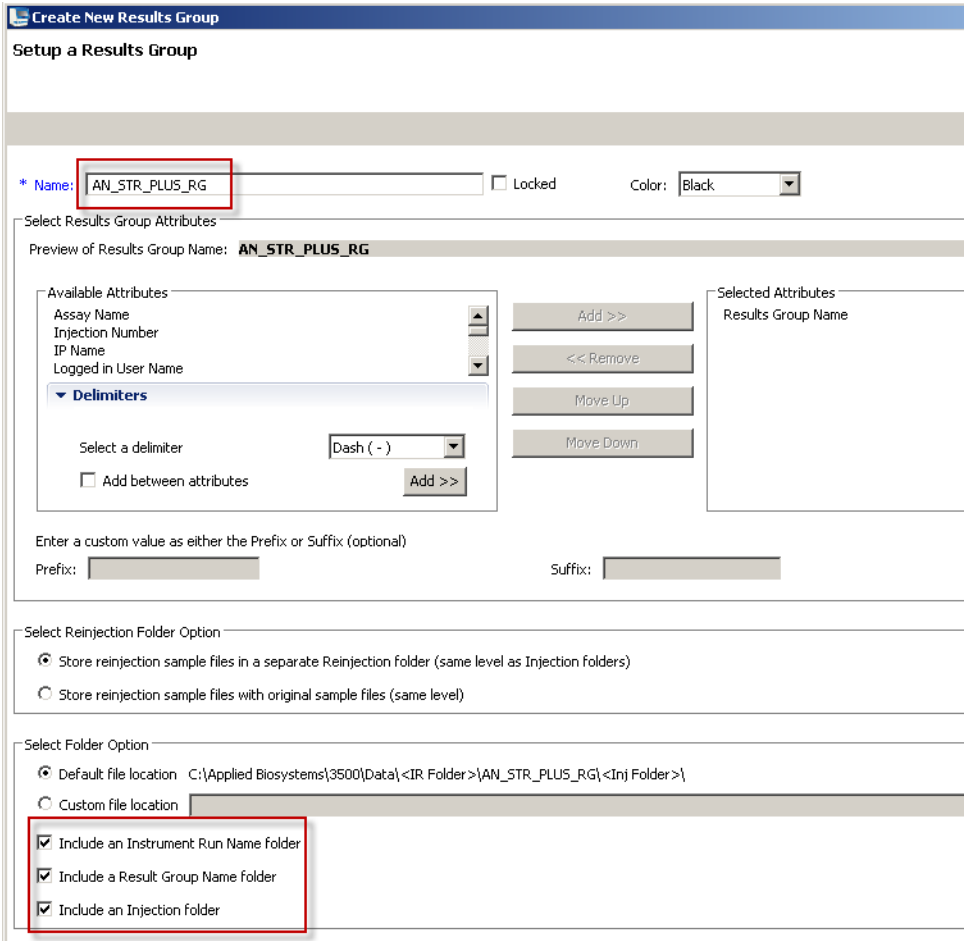
   d. Repeat steps a through c to create additional file name conventions.

8. To create new results groups and add them to the plate template:

   a. Under Results Groups, click **Actions**, then select **New**.

   b. Edit the results group as shown below to store the sample data files in a folder with the results group name.





   c. Click **Apply to Plate** (in addition, you can click **Save to Library**), then click **Close**.

   d. Repeat steps a through c to create additional results groups.

9. In the menu bar, click **Save Plate** ▸ **Save As Template**.
   The template icon is displayed below the plate layout.

For each run: Create a new plate and start the run

After setting up the software (see "Before first run: Set up Data Collection Software" on page 21), use the following instructions each time you load a reaction plate into the 3500 instrument.

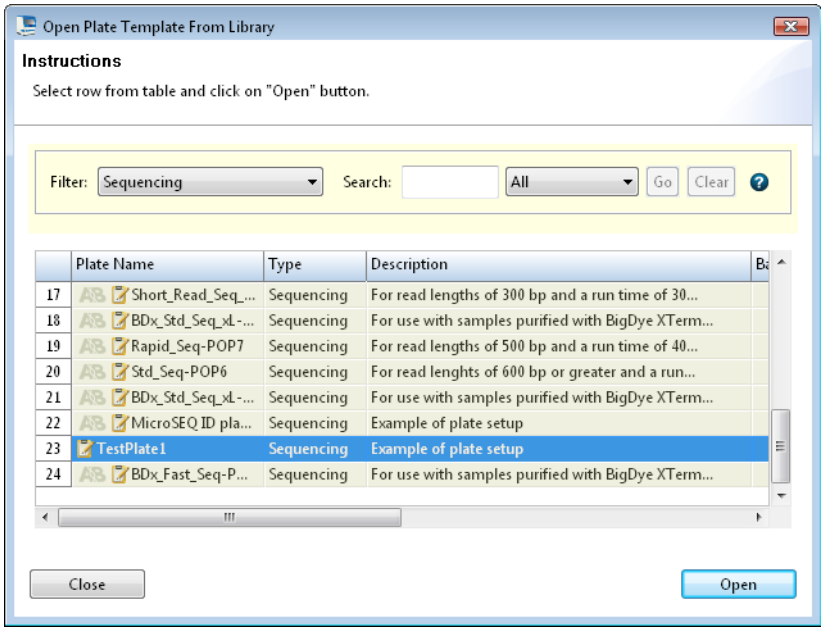
Note: If you want to perform normalization on the 3500 Series Genetic Analyzers, you must use GeneScan™ 600 LIZ™ Size Standard v2.0 (For 3500 Series Normalization) chemistry.

Note: For more information, access the Help system by pressing **F1**, by clicking in the toolbar of the Data Collection Software window, or by selecting **Help ▸ Contents and Index**, or refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (PN 4401661).

Create a new plate from a template

1. In the Dashboard, click 

(Create Plate From Template) to display the Open Plate Template from Library dialog box.



2. Select the template you edited in "Edit the plate template (one-time setup)" on page 22, then click **Open**.

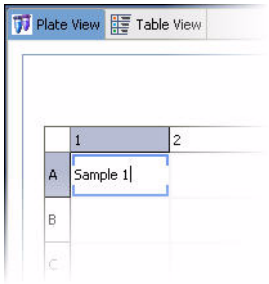
3. In Plate Details, enter a unique plate name. Optionally, enter the Owner, Barcode, and Description.

4. In the left pane, click **Assign Plate Contents**.

5. In the **Assign Plate Contents** screen, select the **Plate View**.

6. Click a well, enter a name for the sample, then press **Enter**. Enter a name for each sample and control in the plate.

   Note: Add PLUS, XY, 21, 18, or 13, as appropriate, to the beginning of each sample name to facilitate sorting in GeneMapper® Software.



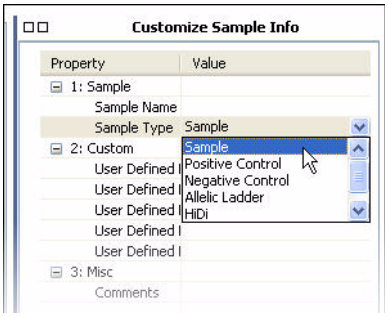
   Note: For options for entering sample information, access the Help system by pressing **F1**, by clicking in the toolbar of the Data Collection Software window, or by selecting **Help ▸ Contents and Index**.

7. Assign a sample type to each sample:

   a. Select all named wells.

   b. In the bottom right of the Assign Plate Contents screen, expand the Customize Sample Information pane.

   c. Specify the sample type for the selected samples and controls, then press **Enter**.

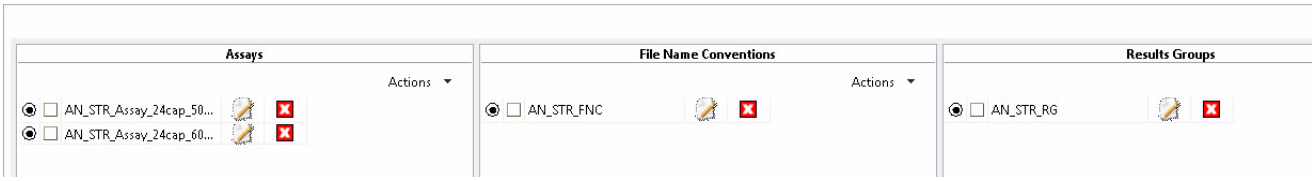


8. Assign the assay, file name convention, and results group to the named wells:

   a. In the Plate View, click-drag to select the wells for which to specify an assay, file name convention, and results group.

b. Enable the checkboxes next to the appropriate assay, the file name convention, and the results group, as shown in the table below. The figure below shows selections for a 24-cap plate.

Assay	For the 3500 instrument: • **AN_STR_Assay_8cap_500LIZ** *or* • **AN_STR_Assay_8cap_600LIZ_Norm** For the 3500xL instrument: • **AN_STR_Assay_24cap_500LIZ** *or* • **AN_STR_Assay_24cap_600LIZ_Norm**
File Name Convention	**AN_STR_FNC** (or the kit-specific file name convention you added to the plate template)
Results Group	**AN_STR_RG** (or the kit-specific file name convention you added to the plate template)



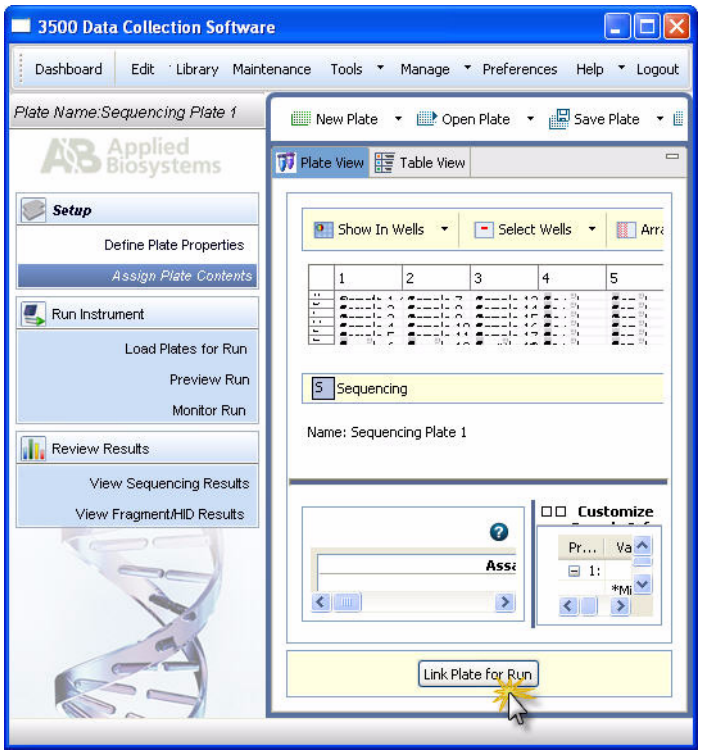
9. Click **Save Plate**.

Link the plate and start the run

Note: Before beginning the run, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (PN 4401661) for instructions on starting the system, logging in, checking the system status, and checking the consumables status.

1. Prepare the plate with the samples, then load it into the instrument autosampler.

2. In the Data Collection Software, in the Assign Plates for Run screen, click **Link Plate for Run**.



3. In the Load Plates for Run screen:
   a. Review the consumables information and the calibration information and ensure the status is acceptable for a run.
   b. Enter a Run Name or use the default run name: <Start Instrument Run Date/ Time Stamp> YYYY-MM-DD-hh-mm-ss-SSS (milliseconds), for example, "Run 2009-02-05-15-03-42-096" where the run start date is February 5, 2009, and the run start time is 15:03:42:096.
   c. Click **Start Run**. The Monitor Run screen is automatically displayed.

After the run, follow the procedures in Chapter 3.

3 Set Up GeneMapper® Software and Perform Data Analysis

This chapter covers:

Workflow for GeneMapper® Software setup and data analysis

Import GeneMapper® Software settings (page 31)

Import analysis settings (one-time setup) (page 31)

▼

Import the panels and bin set (one-time setup) (page 33)

▼

Create a new project and add sample files (page 35)

▼

Apply analysis settings to samples and perform analysis(page 37)

Determine the analysis range for your data (page 37)

▼

Apply and edit the analysis method (page 38)

▼

Apply the panels (page 41)

▼

Apply the size standard (page 42)

▼

Analyze the samples (page 43)

▼

Review the sizing quality values and the size standard (page 44)

Review the sizing quality and contributing PQVs (page 44)

▼

Review and troubleshoot the size standard (page 45)

▼

(Optional) Edit the size standard (page 47)

Review the allele calls and generate a report (page 48)

View the sample plots and review the allele calls for each sample (page 48)

▼

(If necessary) Edit peak labels (page 49)

▼

Review the project samples in a report table (page 50)

GeneMapper® Software terms

Term	Definition
analysis parameters	A collection of user-defined settings (including an analysis method, size standard, and panel) that determine the sizing and genotyping algorithms used by the GeneMapper® Software to analyze all sample files in a project.
bin	A fragment size (in base pairs) and dye color that define an allele within a marker. A bin is created for each possible allele associated with a marker.
bin set	A collection of bins (allele definitions), specific to a set of experimental conditions.
marker	A marker is defined by a name and fragment size range (bp).
panel	A group of markers. In the GeneMapper Software, a panel is associated with a bin set to provide bin definitions for the markers.
kit	A group of panels.

Import GeneMapper® Software settings

Before using GeneMapper® Software for the first time, follow the directions below to:

- Import the recommended Analysis Method, Size Standard Definition, Table Settings, Plot Settings and Report Settings
- Import the recommended panels and bin set

Import analysis settings (one-time setup)

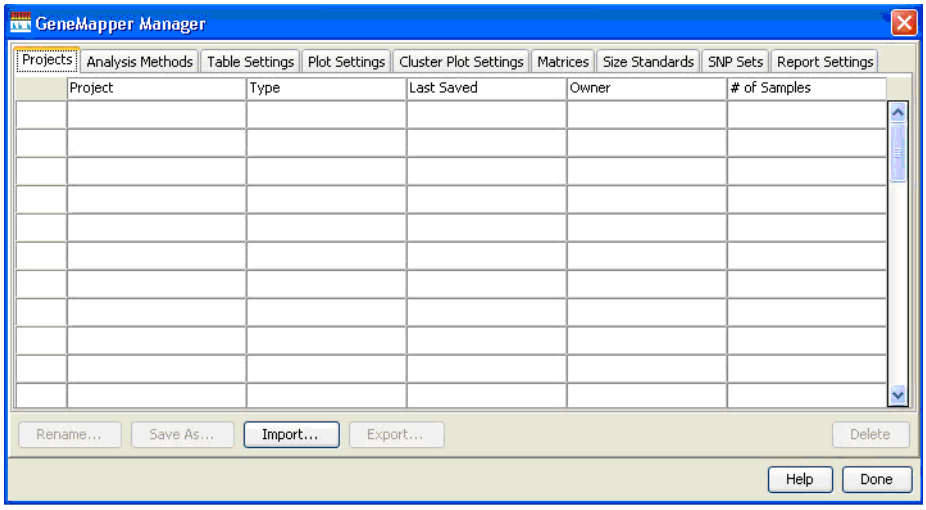
1. Download the appropriate file:
   a. Go to **www.appliedbiosystems.com/aneuploidy**, then select the **Literature/ Support** tab.
   b. Under Software Downloads, click the Download software settings link.
   c. Select the appropriate file for your GeneMapper® Software version:
      - **AN_STR_GMv4_1_Support_Files_Rev1.zip**

      or
      - **AN_STR_GMv4_0_Support_Files_Rev1.zip**
   d. In the File Download dialog box, click **Save**, then save the file to your computer.
   e. Unzip the file.

2. Select **Start ▸ All Programs ▸ Applied Biosystems ▸ GeneMapper ▸ GeneMapper 4.1 (or 4.0) to launch the GeneMapper®** Software.

3. In the Login to GeneMapper dialog box:
   a. Enter the User Name and Password assigned by your system administrator.
   b. Click **OK**.

4. Click (Tools ▸ GeneMapper Manager).



5. Perform steps a to e to import all of the files listed in the table below:
   a. Click the tab shown.
   b. Click **Import** to open the dialog box.
   c. In the dialog box, navigate to the **AN_STR_GMv4_1_Support_Files_Rev1** (or **AN_STR_GMv4_0_Support_Files_Rev1)** folder.
   d. Open the folder and select the file shown in the table.
   e. Click **Import**.

Tab	Dialog Box	File
Analysis Methods	Import Analysis Method†	**AN STR Analysis Method.xml**
Table Settings	Import Table Settings	**AN STR Table Settings.xml**
Plot Settings	Import Plot Settings	**AN STR Plot Settings.xml**
Report Settings	Import Report Settings	**AN STR Report Settings.xml**

† You may need to modify the parameters in the Analysis Method to accommodate your specific sample requirements.

6. Click **Done** to close the GeneMapper Manager.

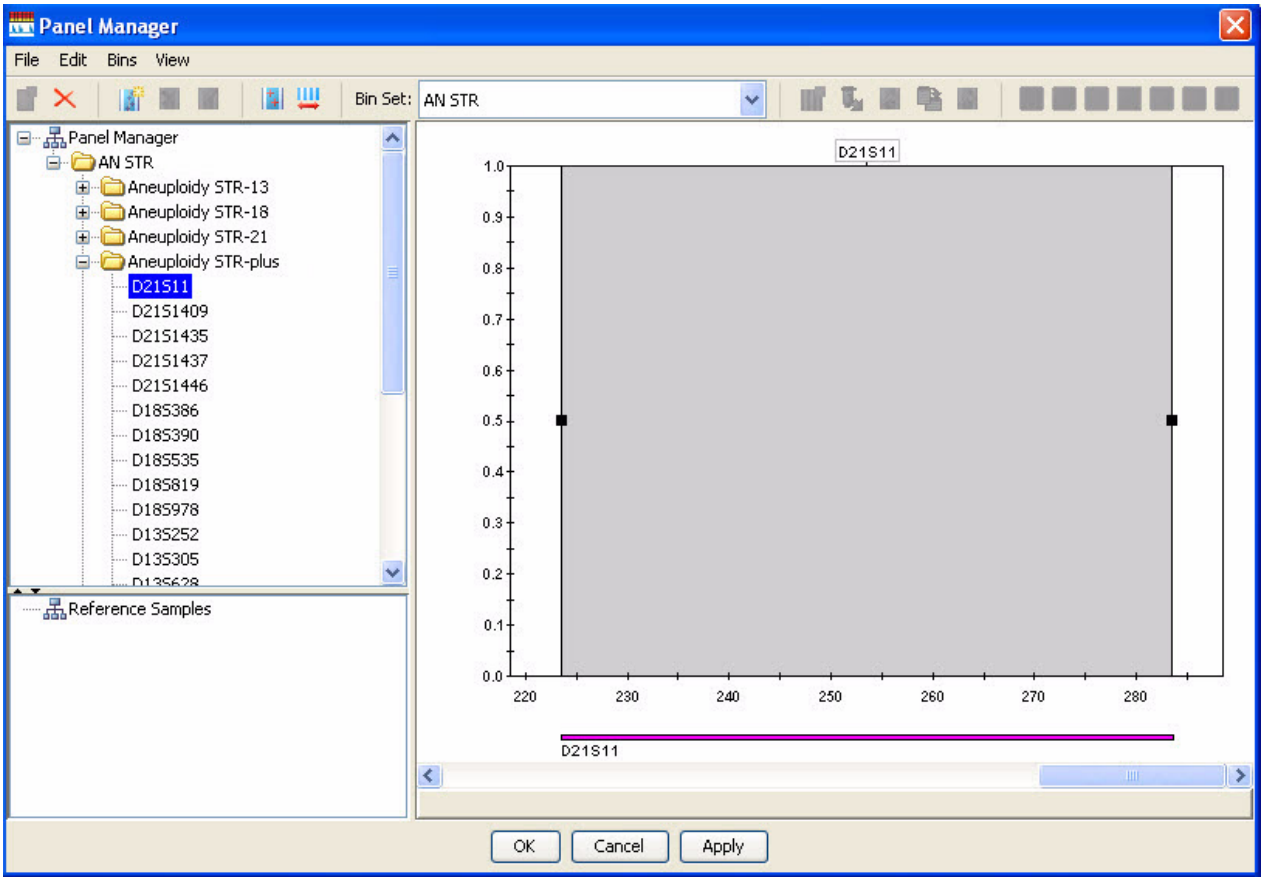
Import the panels and bin set (one-time setup)

1. Import the folder containing the **AN STR** panels:
   a. Click  (Tools ▸ Panel Manager).
   b. Select Panel Manager at the top of the Navigation Pane (left side), then click **File ▸ Import Panels**.
   c. In the Import Panels dialog box, navigate to the **AN_STR_GMv4_1_Support_Files_Rev1** (or **AN_STR_GMv4_0_Support_Files_Rev1)** folder.
   d. Select the **AN STR_Panels.txt** file.
   e. Click **Import**.

      The kit folder containing the imported panel information appears in the Navigation Pane (left side).

2. Import the **AN STR** Bin Set:
   a. In the Panel Manager, highlight the **AN STR** folder in the Navigation Pane (left side), then click **File ▸ Import Bin Set**.
   b. In the Import Bin Set dialog box, navigate to the **AN_STR_GMv4_1_Support_Files_Rev1** (or **AN_STR_GMv4_0_Support_Files_Rev1)** folder.
   c. Select the **AN STR_Bins.txt** file.

d. Click **Import**.

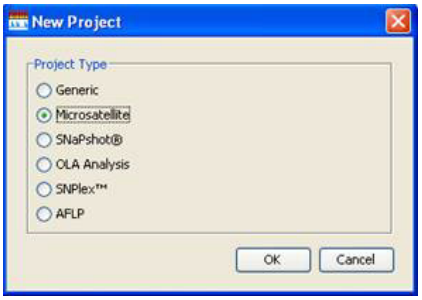


3. Click **OK** to save the settings and close the Panel Manager.

Create a new project and add sample files

Use the following instructions to create a new project for your sample files after each capillary electrophoresis run.

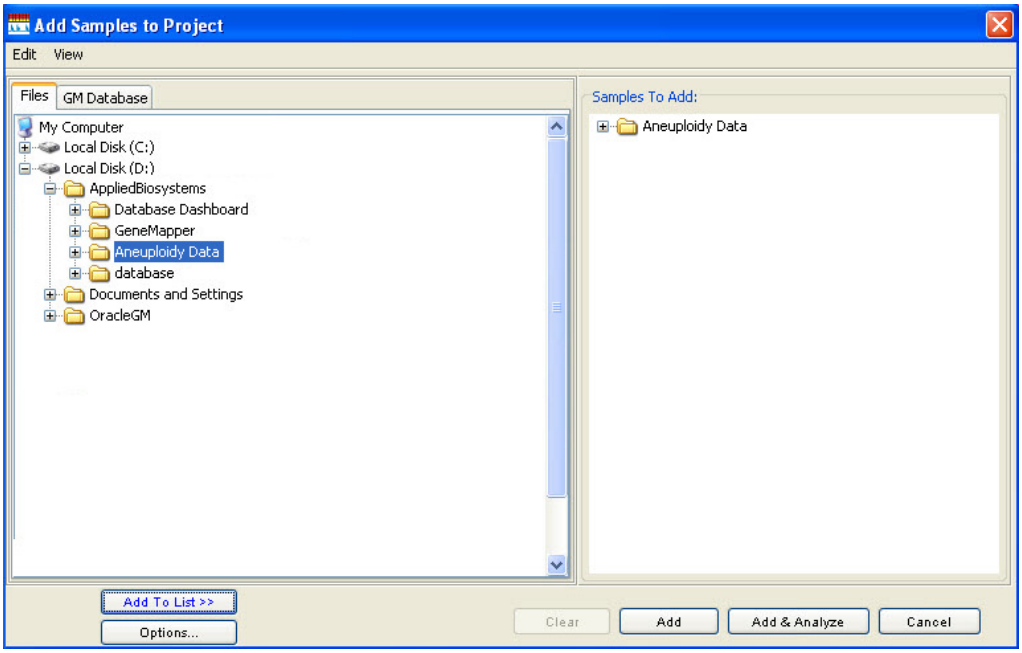
1. If GeneMapper® Software is not already running, select **Start ▸ All Programs ▸ Applied Biosystems ▸ GeneMapper ▸ GeneMapper 4.1 or 4.0**.
2. In the Login to GeneMapper dialog box:
   a. Enter the User Name and Password assigned by your system administrator.
   b. Click **OK**.
3. Click  (File ▸ New Project).
4. In the New Project dialog box, select **Microsatellite**, then click **OK**.



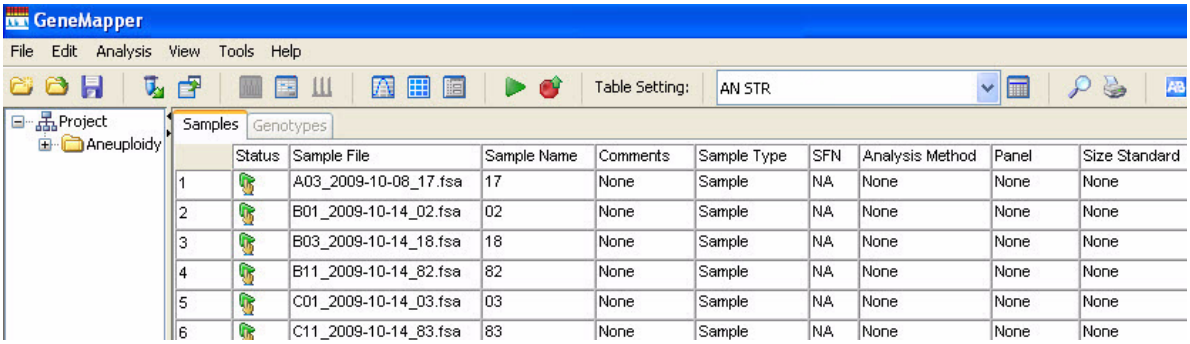
5. From the GeneMapper window, click  (File ▸ Add Samples to Project).
6. In the Add Samples to Project dialog box, in the Files tab, navigate to your sample folder.

   Note: The sample folder location will vary.

7. Select the samples from the folder, click **Add to List**, then click **Add**.



Your sample files will appear in the Samples tab of the Main Project Window:



	Status	Sample File	Sample Name	Comments	Sample Type	SFN	Analysis Method	Panel	Size Standard
1		A03_2009-10-08_17.fsa	17	None	Sample	NA	None	None	None
2		B01_2009-10-14_02.fsa	02	None	Sample	NA	None	None	None
3		B03_2009-10-14_18.fsa	18	None	Sample	NA	None	None	None
4		B11_2009-10-14_82.fsa	82	None	Sample	NA	None	None	None
5		C01_2009-10-14_03.fsa	03	None	Sample	NA	None	None	None
6		C11_2009-10-14_83.fsa	83	None	Sample	NA	None	None	None

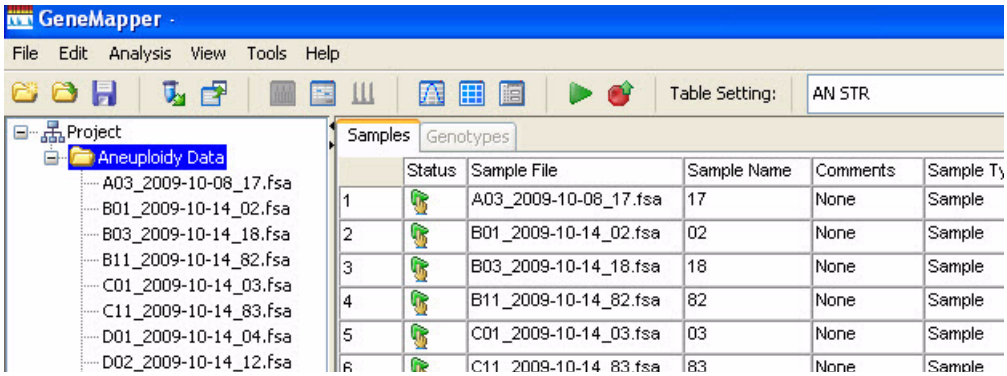
Apply analysis settings to samples and perform analysis

After creating a project and adding sample files, follow these instructions to set the analysis parameters that the GeneMapper Software will use to size and allele-call the data.

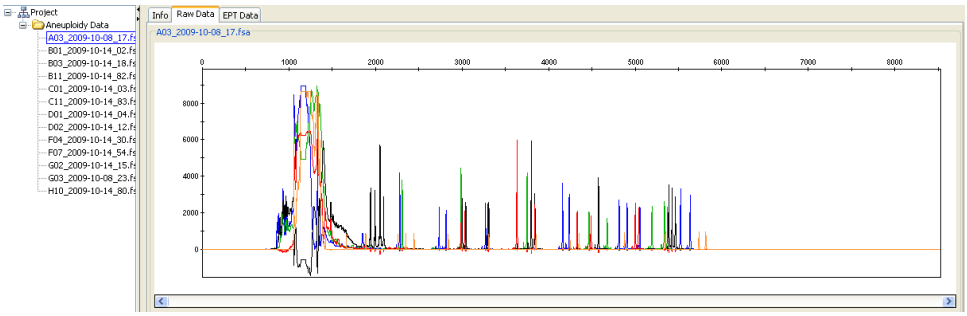
Determine the analysis range for your data

You may need to modify the default Analysis Range in the Peak Detector tab of the Analysis Method to account for variations in run conditions. To determine the analysis range for your data:

1. In the GeneMapper window, expand the run folder in the left navigation pane to show the sample files.

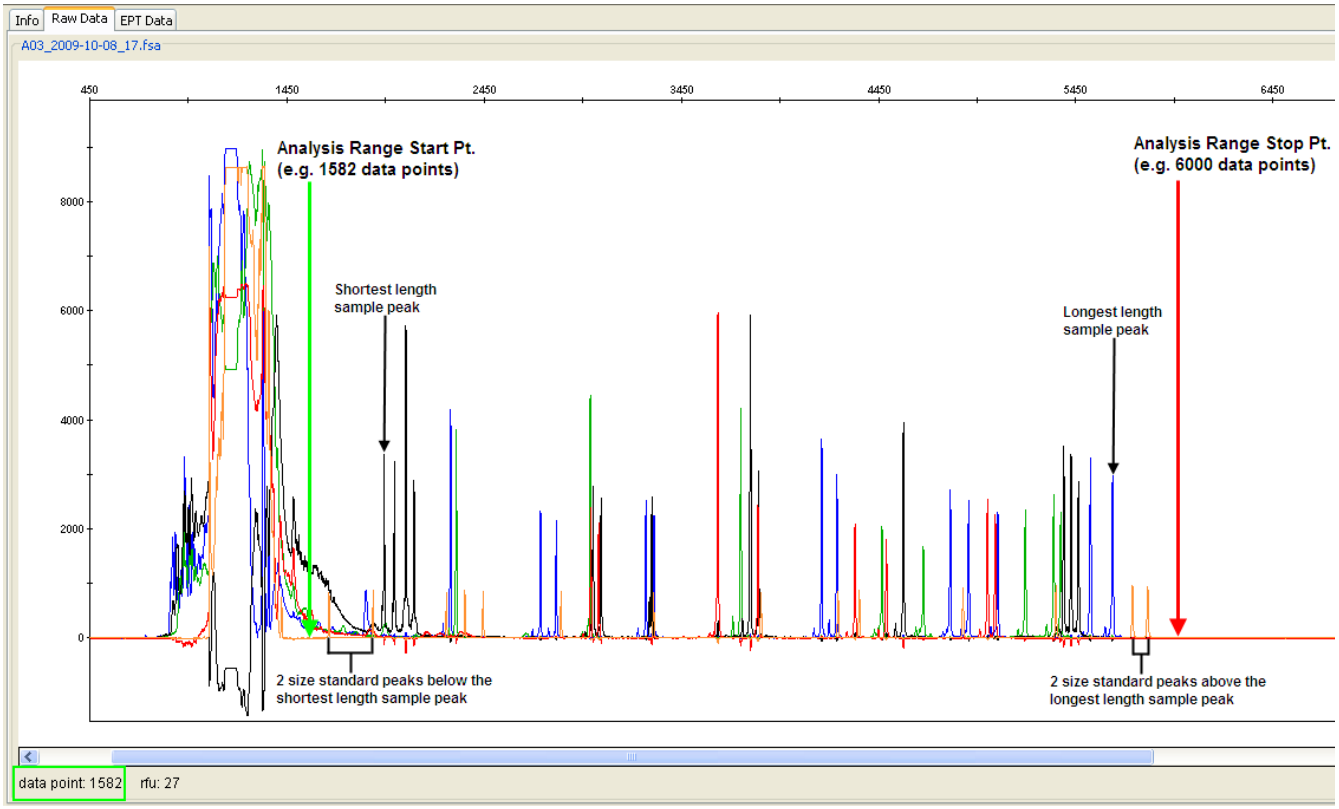


2. Select the first sample file, then click the **Raw data** tab.



3. Using the electropherogram as a guide:
   a. Locate the two size standard peaks below the shortest-length sample peak. Determine the Analysis Range Start by placing the cursor on a point below those two size standard peaks and observing the data point value (lower-left corner).

b. Locate the two size standard peaks above the longest-length sample peak. Determine the Analysis Range End by placing the cursor on a point above those two size standard peaks and observing the data point value (lower left corner).

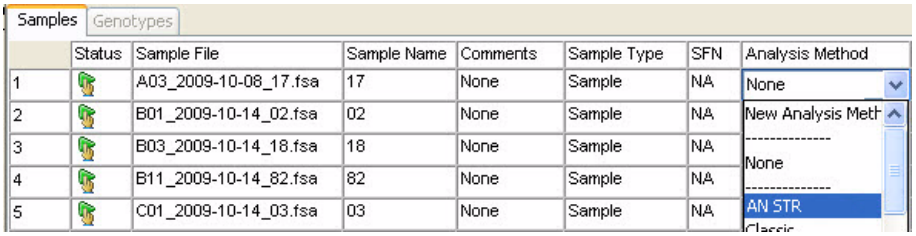


Apply and edit the analysis method

1. In the left navigation pane, select the **Project** folder to return to the main project window.

2. Select the first row in the Analysis Method column.

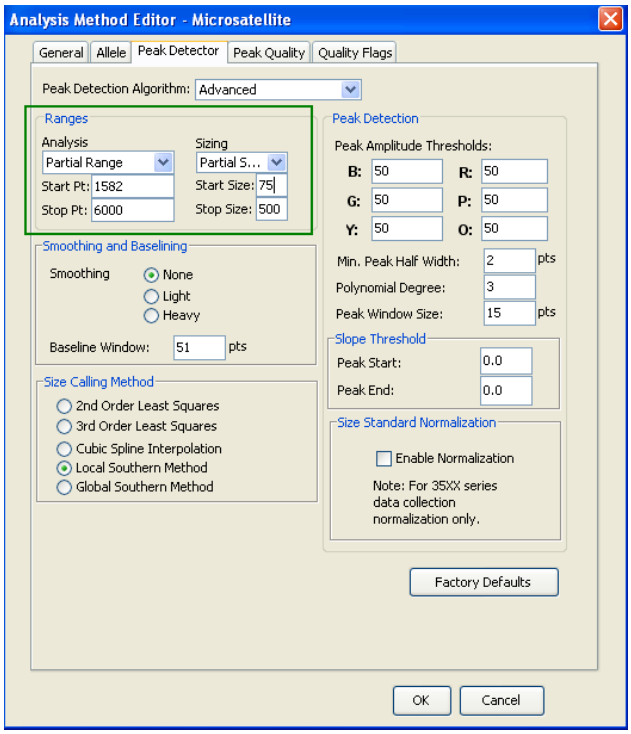
3. Select the **AN STR** analysis method you imported in "Import analysis settings (one-time setup)" on page 31 from the pull-down menu.



4. Double-click the Analysis Method name to open it. (Alternatively, click on [Analysis Method Editor] in the Toolbar.) The Analysis Method Editor opens.

5. In the Analysis Method Editor, select the **Peak Detector** tab, set the Analysis Range to the settings you determined on page 37.

   Note: The Peak Detector tab includes settings that determine peak detection and sizing of peaks.



IMPORTANT! If you modify the Analysis Range, you must modify the Sizing Range (below) and the size standard definition file (see "Apply the size standard" on page 42). The size standard definition file must specify the number of size standard peaks that are present in the Analysis Range. For example, if the Analysis Range analyzes the size standard peaks between 75 bp and 500 bp, you must edit the Sizing Range and the size standard definition for this range.

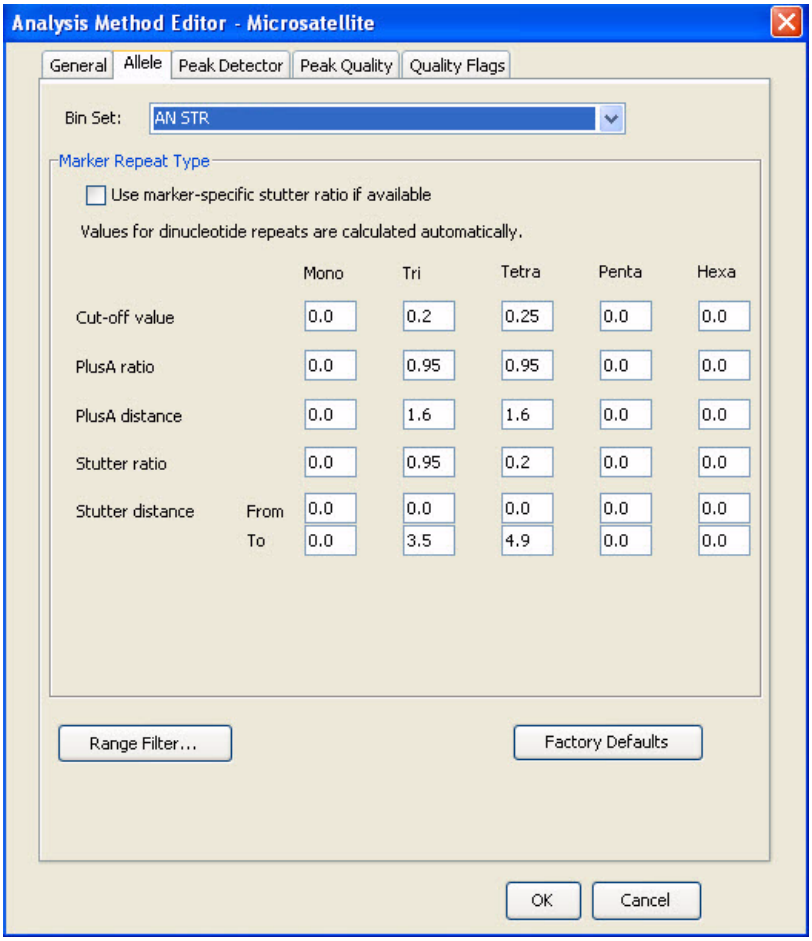
6. (Optional) Use the criteria in the table to help you if you need to modify the Peak Detector tab settings in order to conform to your data requirements.

Table 1 Analysis Method Settings

Parameter	Entry/Selection Criteria
Analysis Range	The data points for the software to analyze. Select the analysis range: • **Full Range**: For the software to analyze all data points. • **Partial Range**: For the software to analyze only data points within a specified range. Enter a Start Pt and a Stop Pt.
Sizing Range	The size range for the software to analyze. Select the sizing range: • **All Sizes**: For the software to analyze fragments of all sizes. • **Partial Sizes**: For the software to analyze only fragments within a specified range. Enter a Start Size and a Stop Size.
Peak Amplitude Thresholds	Only peaks with heights that exceed the peak amplitude threshold value are reported in the table data. For each color, enter a value that allows the software to report peaks and eliminate noise. For example, if you use the default values of 50, peaks with heights above 50 are analyzed and are reported in the tabular data. Peaks with heights below 50 are still displayed in the electropherogram plots but are not analyzed and are not reported in the tabular data. The default peak amplitude threshold values are 50 for the 31XX series Genetic Analyzers and 175 for the 3500 series Genetic Analyzers.
Min. Peak Half Width	Defines what constitutes a peak. Used to specify the smallest full width at half-maximum for peak detection. The range is 2 to 99. Experiment with this value to determine the best number for the data. • Enter a low number if you want the data to display narrow peaks. • Enter a high number if you want to ignore noise spikes.
Polynomial Degree	Sets the degree of the polynomial. Higher degrees increase sensitivity. The range is 2 to 5. • Enter **2** or **3** (default) for well-isolated peaks, such as those from a size standard. • Enter **4** or **5** for finer control.
Peak Window Size	Sets the width of the window. • The minimum value is 1 above the polynomial degree. • The maximum value is the number of data points between peaks. • If you set the polynomial degree to 4, the peak window size should be 1 to 2 times the full width at half-maximum of the peaks you want to detect. • The Peak Window Size setting is limited to odd numbers.
Size Standard Normalization	For data collected from the 3500/3500xL Genetic Analyzer with GS600LIZ™ v.2 Size Standard chemistry, check Enable Normalization to apply the normalization factor to the data. The normalization factor is automatically determined during data collection for all samples collected with the GS600LIZ+Normalization size standard and with a "passed" SQ in the 3500 Data Collection Software.
Factory Defaults	Click Factory Defaults to restore the factory default settings.

7. Select the **Allele** tab.

8. Select the **AN STR** bin set from the Bin Set pull-down menu.

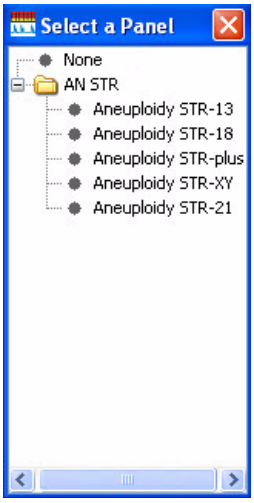


9. Click **OK** to save and close the Analysis Method.

Apply the panels

1. Select the first row in the Panel column. The Select a Panel dialog box appears.

2. From the Select a Panel dialog box, expand the AN STR folder that you imported in "Import the panels and bin set (one-time setup)" on page 33, and then double-click the panel for your data set.



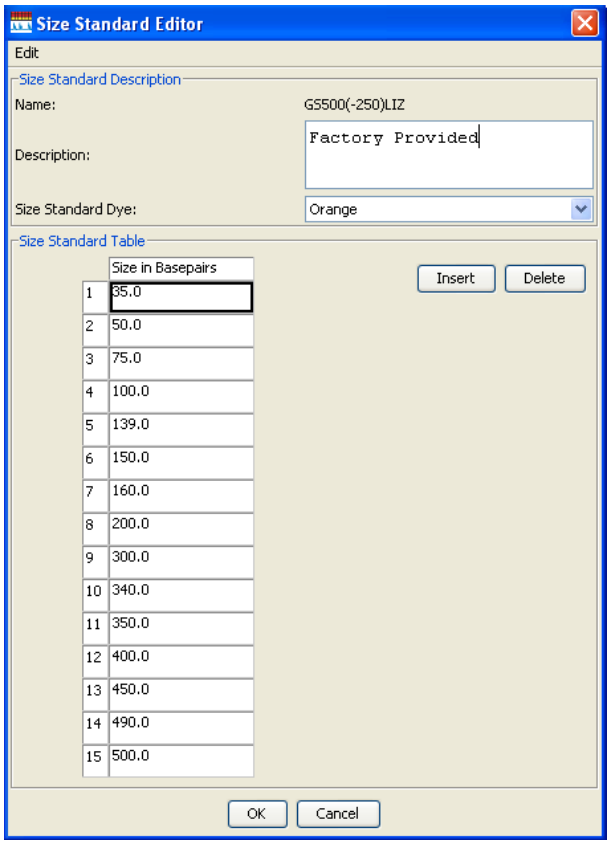
Note: You can analyze data generated for all kits in the same GeneMapper project, but remember to select the appropriate panel for the corresponding data set.

Apply the size standard

1. Select the first row in the Size Standard column.
2. Choose the **GS 500(-250) LIZ**, **GS 600 LIZ,** or **GS 600 LIZ + Normalization** size standard as appropriate for your data set.
3. Double-click the size standard name to open the Size Standard Editor dialog box.

4. Observe that all of the values for the size standard are present and that the dye label is orange, then click **OK**.

   Note: It may become necessary to edit the size standard values in order to conform to your data requirements. See "(Optional) Edit the size standard" on page 47.



Analyze the samples

Fill down the analysis settings

Fill down your selections to all sample rows in the Samples tab:

1. Click-drag across the Analysis Method, Panel, and Size Standard column headers to highlight all rows in all three columns.

2. Select **Edit ▸ Fill Down** (or press Ctrl+D).

Note: You can analyze data generated from multiple kits in the same GeneMapper project, but remember to select the appropriate panel for the corresponding data set. Use the Ctrl key to specifically select the data files for a kit, then press **Ctrl+D** to fill down only the selected cells.
If you added a kit prefix to the beginning of each sample name during your plate setup in data collection, you can sort the samples by panel by holding down **Shift** and clicking the **Sample File** column header.

Analyze the samples

In the Samples tab of the Main Project Window, the icon displays in the Status column, indicating that the samples are ready to be analyzed and have not been analyzed with the current analysis parameters selected in the Samples tab.

Samples | Genotypes

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard
1		A03_2009-10-08_17.fsa	17	Sample	AN STR	Aneuploidy STR-plus	GS500(-250)LIZ
2		B01_2009-10-14_02.fsa	02	Sample	AN STR	Aneuploidy STR-plus	GS500(-250)LIZ
3		B03_2009-10-14_18.fsa	18	Sample	AN STR	Aneuploidy STR-plus	GS500(-250)LIZ
4		B11_2009-10-14_82.fsa	82	Sample	AN STR	Aneuploidy STR-plus	GS500(-250)LIZ
5		C01_2009-10-14_03.fsa	03	Sample	AN STR	Aneuploidy STR-plus	GS500(-250)LIZ
6		C11_2009-10-14_83.fsa	83	Sample	AN STR	Aneuploidy STR-plus	GS500(-250)LIZ

1. Click (Analysis ▸ Analyze).
2. In the Save Project box that opens, enter a project name, then click **OK**.

   The GeneMapper Software analyzes each sample in the project, displaying its progress in the Status Bar (lower left) of the GeneMapper window.

Review the sizing quality values and the size standard

Review the sizing quality and contributing PQVs

1. Make sure "Analysis Completed" appears in the Status Bar (lower left) of the GeneMapper window.
2. Review the sizing quality (SQ) by scrolling to the right in the Samples tab.
   - If the SQ and all associated PQVs (SFNS, SNF, and OS) columns display (Pass), then continue to "Review the allele calls and generate a report" on page 48.
   - If the SQ column displays (Check) or (Low Quality) and the associated PQV columns (SFNF, SNF, and OS) display (indicating issues with the size standard, data, or analysis parameters), investigate and correct these issues. See "Review and troubleshoot the size standard" on page 45.

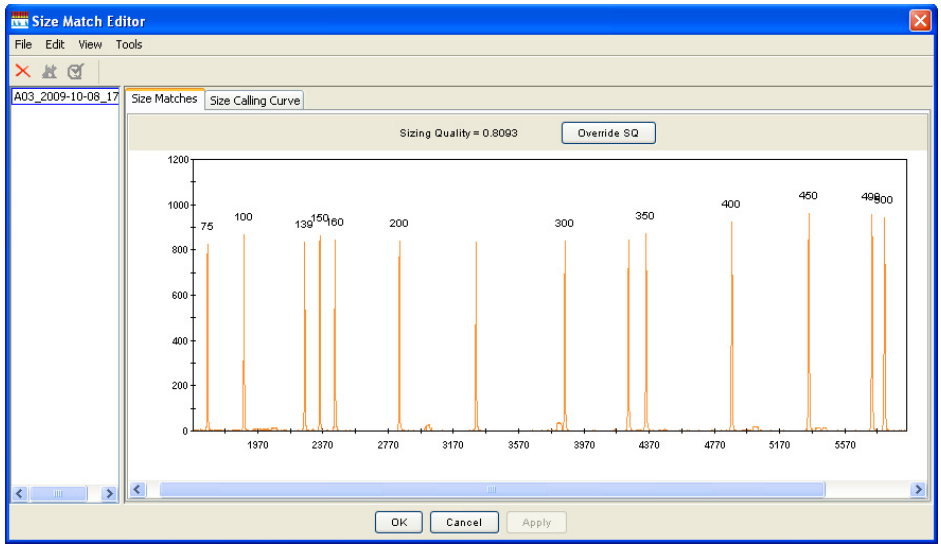
   Note: Click to sort the samples by SQ score. Samples that display a SQ will be listed at the top of the Samples tab.

Review and troubleshoot the size standard

Review the size standard, then troubleshoot and/or edit the size standard if necessary, as described below:

Review the size standard

1. In the Samples tab, select all samples that display (Check) or (Low Quality) SQ by selecting **Edit ▸ Select All**.
2. Open the Size Match Editor by clicking (Analysis ▸ Size Match Editor).

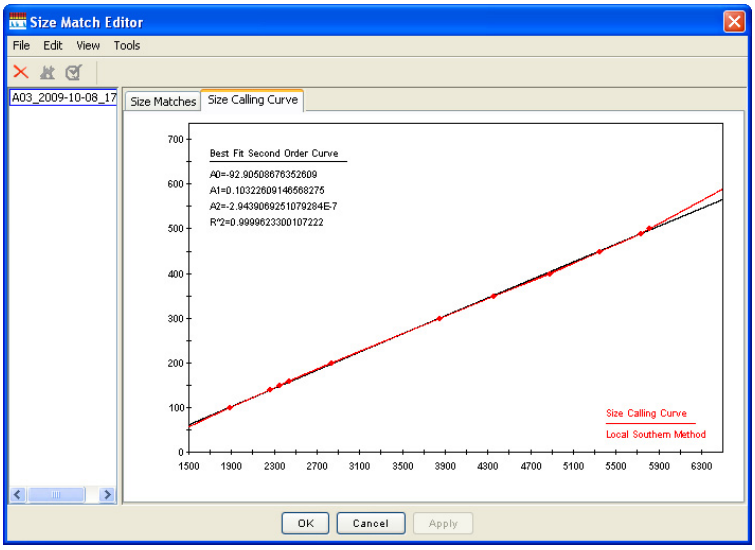


3. Click the Size Matches tab to view the following for the selected sample:
   - Size Quality (SQ) score
   - Size standard peaks
   - Size standard peak labels
4. Note the SQ score for the sample. This score reflects how well the data from the size standard match the size standard definition you selected in the software. This score determines whether the SQ displays (Pass), (Check) or (Low Quality).

   If you used the default Quality Flag settings in this guide, a passing Sizing Quality is > 0.75.
5. Determine if all peaks in the size standard are present and labeled correctly.

   Note: When analyzing your own data you may find some size standards peaks to be incorrectly labeled or missing. For troubleshooting help, see "Troubleshoot the size standard" on page 47.

6. Select the **Size Calling Curve** tab to view the size standard curve for the selected sample. You will see red data points representing the fragments from the size standard and a black best-fit curve.

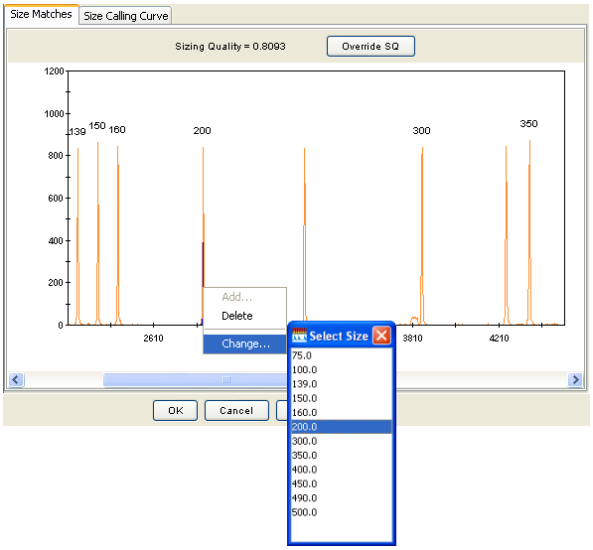


7. Repeat steps 3 to 6 for all samples in your project, if necessary.

8. Click **OK** to close the Size Match Editor.

Troubleshoot the size standard

Table 2 Troubleshoot the size standard

Problem	Action
Sizing Quality score is low and the SQ displays (Check) or (Low Quality), but all size standard peaks are present and labeled correctly.	Override the Sizing Quality by clicking **Override SQ** at the top of the Size Matches tab. Overriding changes the Sizing Quality score to 1.0, indicating the user verified the size standard.
Some size standard peaks are not labeled correctly.	Change, delete, or add size labels in the Size Matches tab. Select the peak, right-click to open the edit menu, make the appropriate edits, then click **Apply** to save the updated sizing information.  You may need to reanalyze the data.
Some size standard peaks are not present.	Create a custom size standard in the software.

Note: For additional help in troubleshooting sizing problems, refer to the *GeneMapper® Software Reference and Troubleshooting Guide* (PN 4403673).

(Optional) Edit the size standard

To edit a size standard and save it under a different name:

1. Navigate to the **GeneMapper Manager** ▸ **Size Standards** tab.
2. Select the size standard you want to edit.
3. Click **Save As** to save the size standard under a different name.
4. Click **Open** and make your edits.
   You can add or remove values from the size standard definition.

Review the allele calls and generate a report

To review the allele calls and generate a report:

1. View the sample plots and review the allele calls for each sample as described on page 48.
2. Review the report table results as described on page 50.

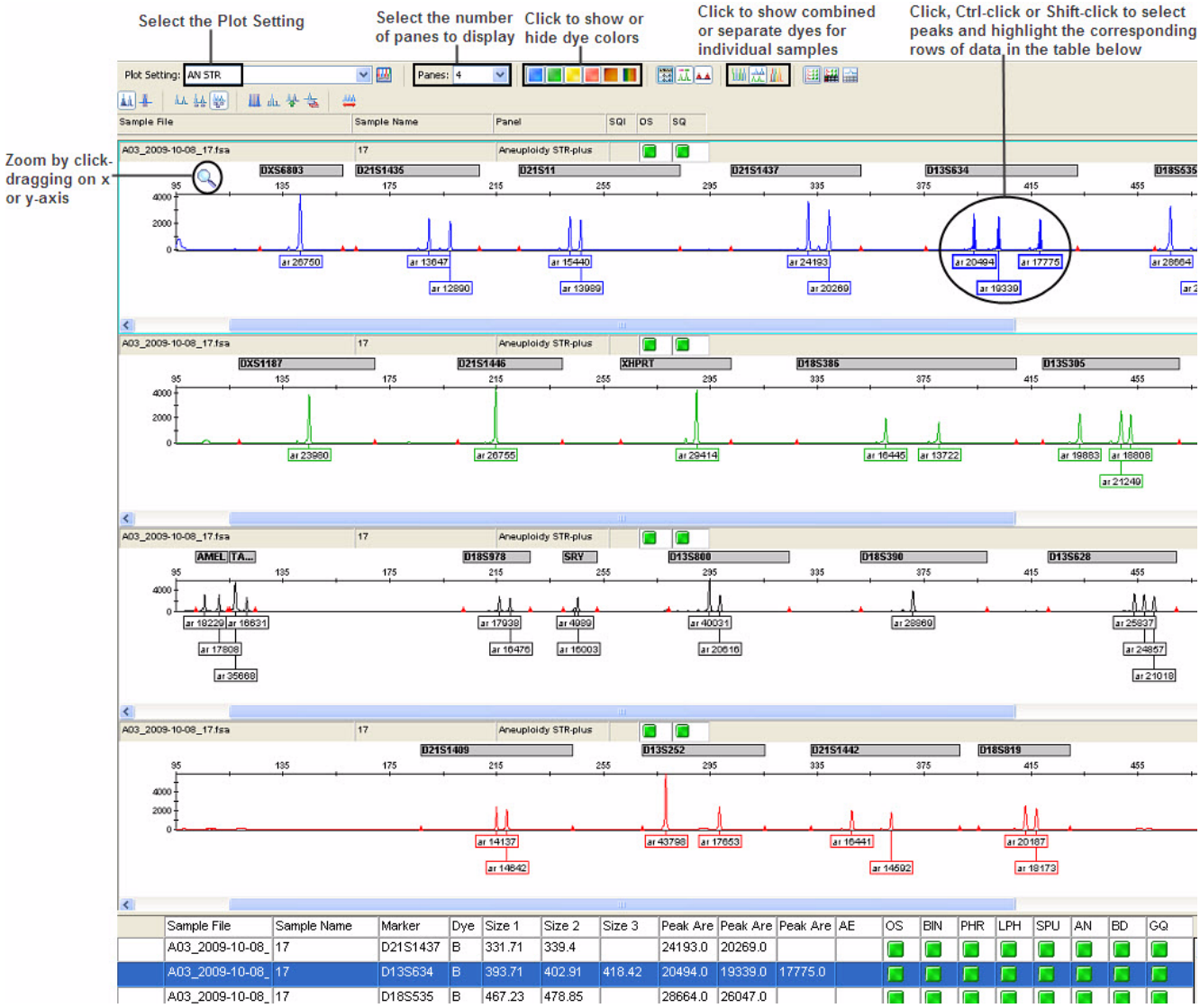
View the sample plots and review the allele calls for each sample

1. Select **View** ▸ **Samples** to display the Samples tab.
2. Select a sample (row) in the Samples tab. To select multiple samples, press and hold Shift or Ctrl. To select all samples, select **Edit** ▸ **Select All**.
3. Click (Analysis ▸ Display Plots), then select **AN STR** in the Plot Setting drop-down list.
4. Review the allele calls for each sample in the Samples Plot window as shown in Figure 1 on page 49.

   Note: If you need instructions on how to modify or delete a label, access the Help system by pressing **F1**, by clicking in the toolbar of the GeneMapper® Software window, or by selecting **Help** ▸ **Contents and Index**.

   Note: In the Samples Plot window, you can:
   - Adjust the scale of the x-axes (basepairs or data points)
   - Adjust the scale of the y-axes (scale to individual maximum, global maximum, or a specific value)
   - Show and hide specific dye color peaks
   - Display a status line for individual peaks
   - Display a Sizing Table, which displays a row of sizing information for each detected peak
   - Display a Genotypes Table, which displays a row of genotyping information for each detected peak, including the allele calls
   - Select peaks, which highlights a corresponding row of data in the corresponding table

Figure 1 The AN STR plot setting displays four panes for each sample you selected in the Samples tab. The Genotypes Table displays the sample information.



Note: Allele size ranges for each marker are based on previously validated data. You may need to adjust the bin set to include rare alleles.

Edit peak labels

You may need to edit peak labels to remove labels from stutter peaks that do not conform to the stutter ratio filters included in the Analysis Method

To modify a peak label, right-click the peak to open the Allele Edit menu, then select one of the options. Adding a comment is optional. Click **OK** to save the edit. The plot and table view immediately update to reflect the changes made.

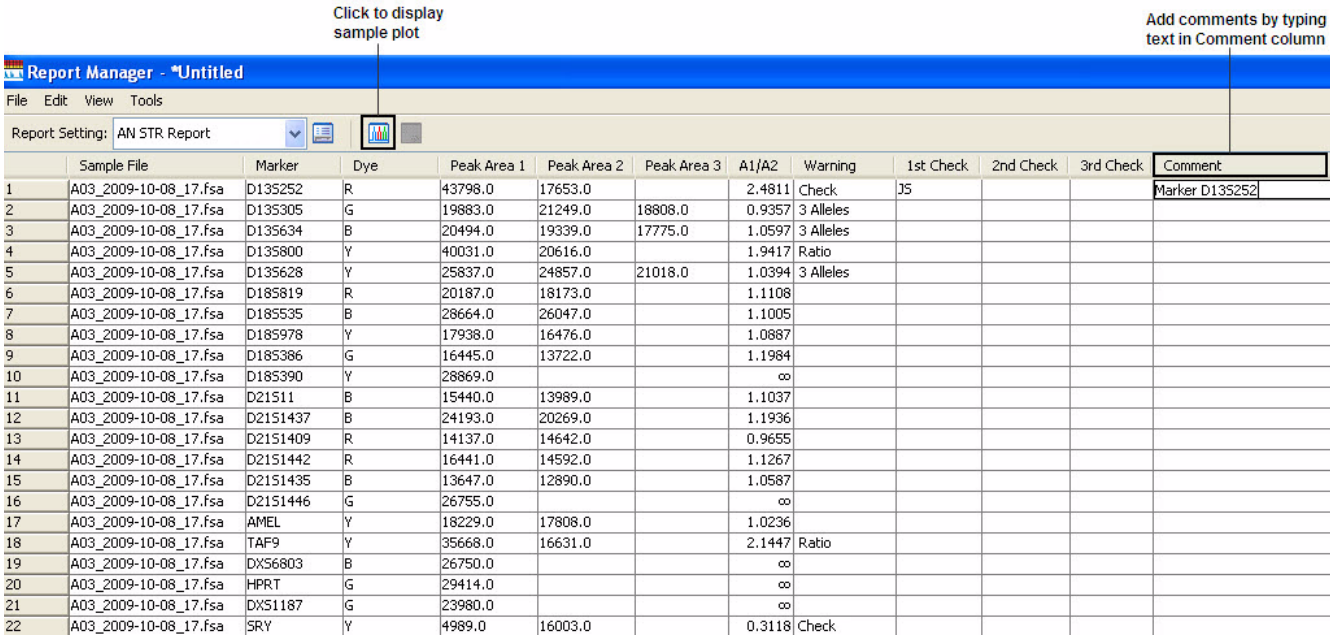
Note: For more information about editing peak labels, access the Help system by pressing **F1**, by clicking in the toolbar of the GeneMapper® Software window, or by selecting **Help ▸ Contents and Index**.

Review the project samples in a report table

1. Select the **Samples** tab and select one sample (row), multiple samples (Shift-click or Ctrl-click) or all samples (Edit ▸ Select All).
2. Select  (Analysis ▸ Report Manager).
3. Select **AN STR Report** from the Report Setting pull-down menu.
4. Review the table results.

   The Report table screens for the following parameters. If they occur, the word in parentheses below is shown in the Warning column.

   - Abnormal 2:1 or 1:2 ratios (Ratio)
   - Ratio calculation results that are between the normal range and the abnormal range (Check)
   - The presence of three alleles within a marker range (3 Alleles)
   - No alleles are present (No Detected Alleles)



	Sample File	Marker	Dye	Peak Area 1	Peak Area 2	Peak Area 3	A1/A2	Warning	1st Check	2nd Check	3rd Check	Comment
1	A03_2009-10-08_17.fsa	D13S252	R	43798.0	17653.0		2.4811	Check	J5			Marker D13S252
2	A03_2009-10-08_17.fsa	D13S305	G	19883.0	21249.0	18808.0	0.9357	3 Alleles				
3	A03_2009-10-08_17.fsa	D13S634	B	20494.0	19339.0	17775.0	1.0597	3 Alleles				
4	A03_2009-10-08_17.fsa	D13S800	Y	40031.0	20616.0		1.9417	Ratio				
5	A03_2009-10-08_17.fsa	D13S628	Y	25837.0	24857.0	21018.0	1.0394	3 Alleles				
6	A03_2009-10-08_17.fsa	D18S819	R	20187.0	18173.0		1.1108					
7	A03_2009-10-08_17.fsa	D18S535	B	28664.0	26047.0		1.1005					
8	A03_2009-10-08_17.fsa	D18S978	Y	17938.0	16476.0		1.0887					
9	A03_2009-10-08_17.fsa	D18S386	G	16445.0	13722.0		1.1984					
10	A03_2009-10-08_17.fsa	D18S390	Y	28869.0			∞					
11	A03_2009-10-08_17.fsa	D21S11	B	15440.0	13989.0		1.1037					
12	A03_2009-10-08_17.fsa	D21S1437	B	24193.0	20269.0		1.1936					
13	A03_2009-10-08_17.fsa	D21S1409	R	14137.0	14642.0		0.9655					
14	A03_2009-10-08_17.fsa	D21S1442	R	16441.0	14592.0		1.1267					
15	A03_2009-10-08_17.fsa	D21S1435	B	13647.0	12890.0		1.0587					
16	A03_2009-10-08_17.fsa	D21S1446	G	26755.0			∞					
17	A03_2009-10-08_17.fsa	AMEL	Y	18229.0	17808.0		1.0236					
18	A03_2009-10-08_17.fsa	TAF9	Y	35668.0	16631.0		2.1447	Ratio				
19	A03_2009-10-08_17.fsa	DXS6803	B	26750.0			∞					
20	A03_2009-10-08_17.fsa	HPRT	G	29414.0			∞					
21	A03_2009-10-08_17.fsa	DXS1187	G	23980.0			∞					
22	A03_2009-10-08_17.fsa	SRY	Y	4989.0	16003.0		0.3118	Check				

IMPORTANT! This report is intended to alert you to samples that require further investigation. You should always review the results visually (see "View the sample plots and review the allele calls for each sample" on page 48) in addition to using the report generation function.

Save, export, or print a report table

After you review the report table results, you can save, export, or print the report.

Save a report table

1. While still in the Report Manager, select **File ▸ Save As**.

2. Enter a unique name for the report and click **OK**.

Note: Saved reports can be accessed by selecting  (Analysis ▸ Report Manager), then selecting **File ▸ Open**.

Export a report table

1. While still in the Report Manager, select **File ▸ Export**.
2. Browse to the folder where you want to export the report, enter a unique name for the report, then click **OK**.

Note: Reports can be exported in .txt and .csv format.

Print a report table

1. While still in the Report Manager, select **File ▸ Print**.
2. Select the settings in the General, Page Setup, and Appearance tabs to customize your print job, then click **Print**.

Note: Reports can be printed to Portable Document Format (PDF).

Documentation and Support

Related documentation

This guide and the following related document are available at:
www.appliedbiosystems.com/aneuploidy

Document	Part number	Description
Applied Biosystems® TrueScience™ Aneuploidy STR Kits Protocol	4454039	Provides recommended instructions for using the Aneuploidy STR Kits from sample preparation through data analysis.

The following documents are referenced in this guide:

Document	Part number	Description
Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide	4401661	Provides step-by-step procedures designed to help you quickly learn to use the 3500 Series instruments.
Applied Biosystems® 3130/3130xl Genetic Analyzers Getting Started Guide	4352715	Provides step-by-step procedures designed to help you quickly learn to use the 3130 Series instruments.
GeneMapper® Software v4.1 Reference and Troubleshooting Guide	4403673	Provides reference and troubleshooting information for GeneMapper® Software.
GeneMapper® Software *v4.1 Installation and Administration Guide*	4403614	Provides installation and administration information for GeneMapper® Software.

Note: To open the user documentation provided in PDF format, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

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